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Evolution of Chemotactic-Signal Transducers in Enteric Bacteria

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The methyl-accepting chemotactic-signal transducers of the enteric bacteria are transmembrane proteins that consist of a periplasmic receptor domain and a cytoplasmic signaling domain. To study their evolution, transducer genes from *Enterobacter aerogenes* and *Klebsiella pneumoniae* were compared with transducer genes from *Escherichia coli* and *Salmonella typhimurium*. There are at least two functional transducer genes in the nonmotile species *K. pneumoniae*, one of which complements the defect in serine taxis of an *E. coli tsr* mutant. The *tse* (taxis to serine) gene of *E. aerogenes* also complements an *E. coli tsr* mutant; the *tas* (taxis to aspartate) gene of *E. aerogenes* complements the defect in aspartate taxis, but not the defect in maltose taxis, of an *E. coli tar* mutant. The sequence was determined for 5 kilobases of *E. aerogenes* DNA containing a 3′ fragment of the *cheA* gene, *cheW*, *tse*, *tas*, and a 5′ fragment of the *cheR* gene. The *tse* and *tas* genes are in one operon, unlike *tsr* and *tar*. The cytoplasmic domains of Tse and Tas are very similar to those of *E. coli* and *S. typhimurium* transducers. The periplasmic domain of Tse is homologous to that of Tsr, but Tas and Tar are much less similar in this region. However, several short sequences are conserved in the periplasmic domains of Tsr, Tar, Tse, and Tas but not of Tap and Trg, transducers that do not bind amino acids. These conserved regions include residues implicated in amino-acid binding.

The methyl-accepting chemotaxis proteins are the best-characterized signal transducers of the chemosensory apparatus in *Escherichia coli* and *Salmonella typhimurium*. Homologous proteins are found in a wide range of bacterial species (35).

Four transducers have been characterized in *E. coli*, and the genes encoding them have been sequenced (5, 6, 24). The Tsr transducer (taxis to serine and away from some repellents) is the receptor for the attractant L-serine and related amino acids and is responsible for chemotaxis away from a wide range of repellents, including leucine, indole, and weak acids (41, 44). Tsr also is involved in thermotaxis (26). The Tar transducer (taxis to aspartate and away from some repellents) is the receptor for L-aspartate and related amino and dicarboxylic acids; Tar also mediates taxis to the attractant maltose via an interaction with the periplasmic maltose-binding protein (14) and taxis away from the repellents Co^{2+} and Ni^{2+} (41). The Trg transducer (taxis to ribose and galactose) interacts with the periplasmic ribose- and galactose-binding proteins to accomplish chemotaxis toward those two sugars (15, 21). The Tap transducer (taxis-associated protein) (7) mediates taxis toward dipeptides via an interaction with the periplasmic dipeptide-binding protein (28; V. Blank, diploma thesis, University of Konstanz, Federal Republic of Germany, 1987).

All transducers characterized to date are composed of the same structural domains (24). The amino terminus resembles the signal peptide of exported proteins, but it is not removed from the mature protein and serves as the first membrane-spanning region. The next ca. 160 amino acids extend into the periplasm, where they form the receptor domain (23). After a second membrane-spanning region, which seems to act as a stop transfer signal (27), the final 300 or more amino acids are localized in the cytoplasm. This portion of the protein generates the intracellular signals to the flagella (32, 36) and contains the glutamic acid residues that are methylated and demethylated during adaptation to chemotactic stimuli (19, 42). The amino acid sequence in regions containing the methylation sites and signaling domain is highly conserved among the four transducers (5, 24).

The genes encoding the signal transducers are located at different points on the *E. coli* chromosome (1); *tsr* at 99 min, *tar* and *tap* together in the *meche* operon at 41 min, and *trg* at 31 min. The genes are part of the flagellar regulon (20), and their promoters contain the consensus sequence present in flagellar operons that require an alternate σ factor for expression (2, 16, 17).

The *tar* gene of *S. typhimurium* also has been sequenced (37). *S. typhimurium* Tar mediates aspartate but not maltose taxis (9, 31). *S. typhimurium* also lacks the Tap transducer and therefore shows no taxis toward dipeptides (28). *S. typhimurium* does respond to serine, ribose, and galactose, so it probably has transducers equivalent to Tsr and Trg. *Enterobacter aerogenes* and *Klebsiella pneumoniae* are somewhat more distantly related to *E. coli* and *S. typhimurium* than the last two species are to each other (38). *E. aerogenes* exhibits chemotaxis to aspartate, maltose, and serine (9; M. Dahl, diploma thesis, University of Konstanz, Federal Republic of Germany, 1985), whereas *K. pneumoniae* is nonmotile.

This work was initiated on the premise that comparison of the transducers from the enteric bacteria will contribute to the study of these proteins. Genes encoding functional transducers were identified in phage λ libraries of chromosomal DNA from *E. aerogenes* and *K. pneumoniae*. We sequenced 5 kilobases (kb) of DNA that encodes serine and aspartate transducers and adjacent chemotaxis-related genes. Analysis of this DNA sequence and of the predicted amino acid sequences derived from it provides information about the function and evolution of proteins involved in chemosensing.

MATERIALS AND METHODS

Reagents. Restriction endonucleases, T4-DNA ligase, Klenow enzyme (DNA polymerase I large fragment), the kit for

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TABLE 1. Strains and plasmids

Strain	Known markers and properties	Source or reference
Bacterial strains		
E. coli		
LE392	F⁻ *supF supE hsdR galK trpR metB tonA*	39
LE392.23	LE392 Δ(*argF-lac*)*U169*	39
RM41	Q358 F⁻ (r⁻ m⁺) *supE* ϕ80ʳ	18
RM42	Q359 P2 lysogen	18
TG1	*pro thi hsd* (r⁻) *lac*/F′ *lacI*ᵠ *lacZΔM15*	8
VB12	*ara-14* Δ(*argF-lac*)*U169 his-4 leuB6 metF159*$_{am}$ *mtl-1 rpsL136* Δ(*tar-tap*)*5201 thi-1 tonA31* Δ*tsr7021 xyl-15*	28
E. aerogenes ATCC 13048	Wild type	13
K. pneumoniae KAY2026	Wild type	40
Phage		
M13mp10	M13 sequencing vector	29
M13mp18	M13 sequencing vector	34
M13mp19	M13 sequencing vector	34
λgt4-lac5	*c*I857(Ts), *lacZ*⁺ *lacY*⁺, helper phage for lysogenizing with λSE6	10
λSE6	Kanʳ, low-copy-number phasmid	11
λSE6-M1	λSE6 carrying *E. aerogenes* DNA containing *'cheA*, *cheW*, *tse*, *tas*, and *cheR'*	This study
Plasmids		
pBR322	Apʳ Tcʳ	4
pJFG5	pBR322 carrying *E. coli tsr*	12
pRK41	pBR322 with 2.4-kb *Cla*I fragment from *S. typhimurium* carrying *tar*$_s$	37
pMK1	pBR322 with 3.3-kb *Eco*RI-*Ava*I fragment with *E. coli 'cheA*, *cheW*, *tar*, and *tap'*	22
pMD5	pBR322 with 5.0-kb *Nru*I fragment from λSE6-M1 carrying *'cheA*, *cheW*, *tse*, *tas*, and *cheR'* from *E. aerogenes*	This study
pMD6	pBR322 with the 3.5-kb *Nde*I fragment from pRK41 and the 1.8-kb *Nde*I fragment from pMK1	This study
pMD7	pBR322 with the 3.2-kb *Nde*I fragment from pRK41 and the 4.5-kb *Nde*I fragment from pMK1	This study
pMD8	pBR322 with the 2.3-kb *Eco*RI-*Stu*I fragment from pMD5 carrying *'cheW*, *tse*, and *tas'*	This study

in vitro packaging of phage λ DNA, and reagents for the M13 dideoxy nucleotide sequencing system were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Isopropyl-1-thio-β-D-galactopyranoside was purchased from Serva. The *lacZ* oligonucleotide primer (17-mer) was obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md., or Boehringer. Deoxy-adenosine-5′-α-[^{35}S]thiotriphosphate was purchased from Amersham Corp., Arlington Heights, Illinois. All other chemicals were of reagent grade.

Strains and plasmids. The bacterial and phage strains and plasmids used in this work are listed in Table 1. *E. coli* VB12 (Δ*tsr7021* Δ*tar-tap5201*) is defective in serine, aspartate, maltose, and dipeptide taxis because of the deletion of the three transducer genes. This strain does not form normal swarms in tryptone soft agar (41) because its steady-state run-tumble behavior is strongly skewed toward running due to the loss of signal input from the missing transducers.

Media and swarm plates. Cells were grown in Luria broth (LB) or minimal medium A supplemented with 0.2% (wt/vol) carbon source, required amino acids at 100 μg/ml, and ampicillin or kanamycin at 50 μg/ml as needed. Media were prepared by the method of Miller (30). Tryptone swarm plates contained 0.3% Bacto-Agar (Difco Laboratories, Detroit, Mich.), 1% Bacto-Tryptone (Difco), and 0.8% NaCl. Minimal swarm plates contained 0.3% Bacto-Agar, motility salts [10 mM potassium phosphate (pH 7.0), 1 mM $(NH_4)_2SO_4$, 1 mM $MgSO_4$, 0.5% (wt/vol) NaCl, and 0.5 μg of $FeCl_3$ per ml], and 100 μM maltose, L-aspartate, or L-serine. Aspartate and serine swarm plates also contained 1 mM glycerol. Ampicillin or kanamycin was added to swarm plates to a final concentration of 50 μg/ml. The plates were scored after 12 to 20 h of incubation at 32°C.

Cloning of genes coding for chemotactic-signal transducers from *E. aerogenes* and *K. pneumoniae*. Chromosomal DNA from *E. aerogenes* ATCC 13048 and *K. pneumoniae* KAY2026 was prepared as described previously (39). Genomic libraries were constructed in phage λSE6 by the method of Elledge and Walker (11), using the *E. coli* strains RM41 and RM42. These libraries were used to infect strain VB12 (Δ*tsr* Δ*tar-tap*) lysogenized with the helper phage λgt4-*lac5*, and a 100-μl sample of washed, infected cells (ca. 5×10^8 cells) was applied in a 5-cm-long trough on swarm plates containing kanamycin. With the *E. aerogenes* library, minimal-aspartate swarm plates were used. With the *K. pneumoniae* library, tryptone swarm plates were used. Within 24 h of incubation at 32°C in minimal agar or 16 h in tryptone agar, chemotactic swarms spreading away from the trough appeared on some of the plates. Single colonies were isolated from these swarms on LB-kanamycin plates, and phage lysates were prepared from well-aerated cultures of these cells growing in LB medium. Phage production was induced by a 15-min shift from 32°C to 42°C, and lysis of the cells proceeded during continued incubation of the cultures

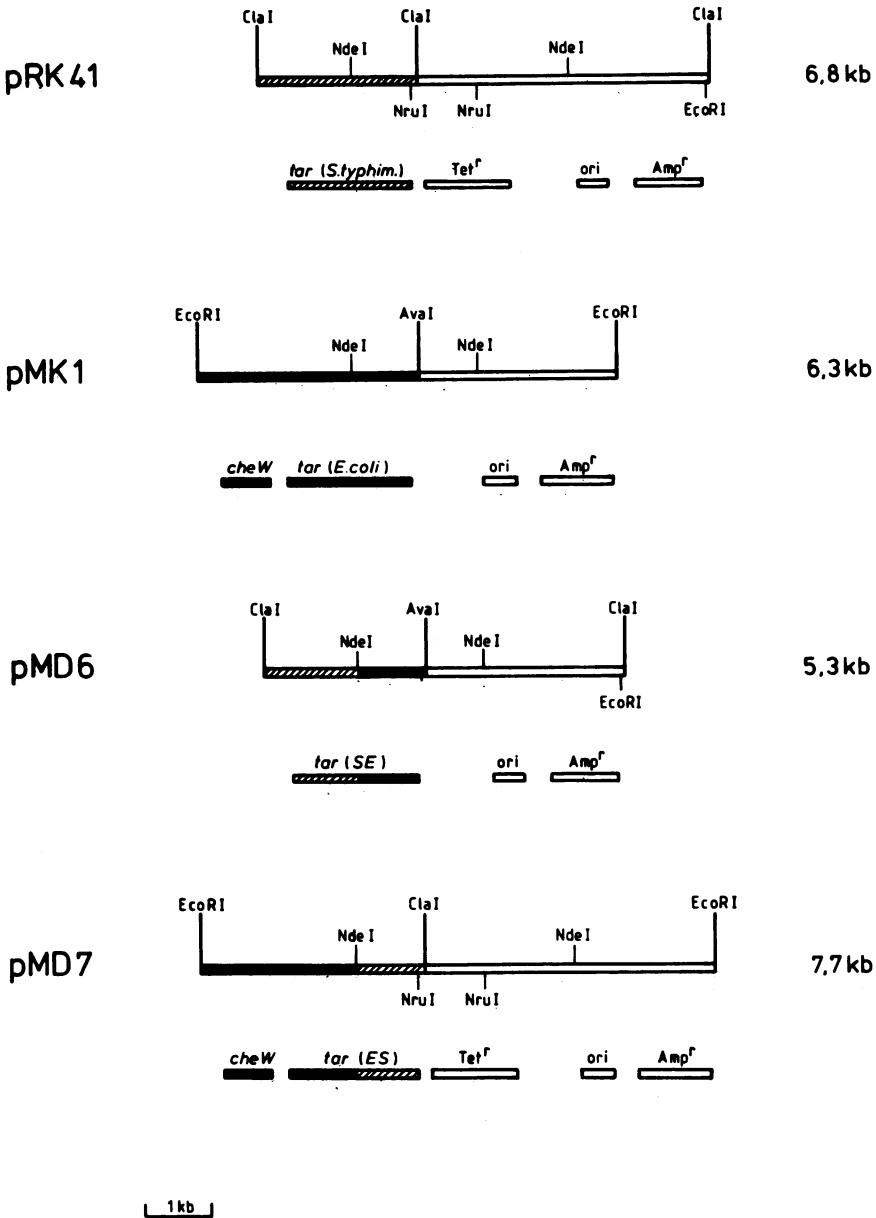

FIG. 1. Construction of *E. coli-S. typhimurium* hybrid *tar* genes. Plasmid pMD6 was made by ligating the 3.5-kb *Nde*I fragment of pRK41 that contains the 5′ end of *S. typhimurium tar* with the 1.8-kb *Nde*I fragment from plasmid pMK1 that contains the 3′ end of *E. coli tar*. Plasmid pMD7 was made by ligating the 4.5-kb *Nde*I fragment of pMK1 that contains the 5′ end of *E. coli tar* with the 3.2-kb *Nde*I fragment from pRK41 that contains the 3′ end of *S. typhimurium tar*. *E. coli* DNA is indicated by black bars, *S. typhimurium* DNA is indicated by hatched bars, and pBR322 DNA is indicated by white bars. The genes carried by each plasmid are indicated below the corresponding restriction map.

at 38°C. The λSE6 and helper phage were separated by plating dilutions of these lysates with *E. coli* LE392.23 on agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside; λSE6 forms white plaques under these conditions, whereas λgt4-*lac5* forms blue plaques.

From the *E. aerogenes* library, 10 independently isolated, plaque-purified phages were retested to confirm that they conferred an aspartate-chemotaxis-positive phenotype upon infecting strain VB12. One of these phages, λSE6-M1, was chosen for further analysis. From the *K. pneumoniae* library, 20 plaque-purified isolates were tested for their ability to complement mutations in *E. coli* transducer genes. Phage λ lysates and DNA were prepared by the methods of Silhavy et al. (39).

Determination of nucleotide sequence. DNA sequencing was performed by the modified dideoxy-chain termination method of Biggin et al. (3) with deoxyadenosine-5′-α-[^{35}S]thiotriphosphate. DNA restriction fragments for sequencing were produced by digesting the purified 5-kb *Nru*I fragment of plasmid pMD5 with the enzymes *Bam*HI, *Cla*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*cII, *Hin*dIII, *Pvu*II, *Sau*3A, *Sma*I, and *Stu*I. These fragments were ligated into the polycloning sites of DNA from phage M13mp10, M13mp18, and M13mp19 digested with appropriate enzymes. M13 phage were propagated on *E. coli* TG1.

Computer analysis. The management of sequence information and the subsequent analysis of compiled data were done with the Macintosh DNA Inspector II program (Microsoft

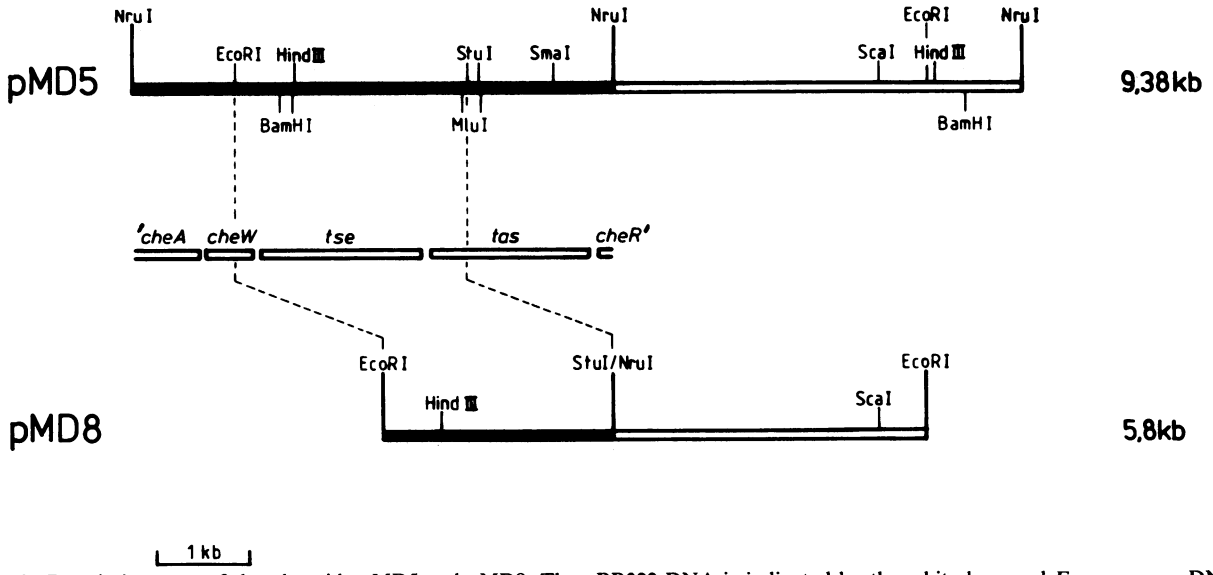

FIG. 2. Restriction map of the plasmids pMD5 and pMD8. The pBR322 DNA is indicated by the white bar, and *E. aerogenes* DNA is shown as a black bar. The genes present on the plasmids are shown between the two restrictions maps.

Basic Runtime, version 2.11; Microsoft Corp.). The DNA Translate program (Mac 1.25) was kindly provided by J. S. Parkinson (Biology Department, University of Utah).

RESULTS

Maltose taxis in cells making hybrid Tar proteins. The difference in the abilities of *E. coli* and *S. typhimurium* to sense maltose is logically attributed to differences in the periplasmic domains of Tar from the two species (9, 31), but this suspicion had not been confirmed experimentally. We decided to utilize chimeric transducers, which were previously used to localize functional domains within the proteins (23), to test this inference.

Construction of the hybrid genes (Fig. 1) took advantage of the *NdeI* site in the *tar* genes from *E. coli* and *S. typhimurium*. Plasmid pMD6 codes for a protein containing the N-terminal 256 residues from *S. typhimurium* Tar and the C-terminal 297 residues from *E. coli* Tar, whereas the protein coded by plasmid pMD7 has the N-terminal 256 residues from *E. coli* Tar and the C-terminal 296 residues from *S. typhimurium*. These two plasmids were transformed into *E. coli* VB12 (*Δtsr7021 Δtar-tap5201*), and the transformants were tested on minimal-aspartate and minimal-maltose swarm plates containing ampicillin.

Cells containing pMD6 formed normal swarm rings only with aspartate, whereas strains containing pMD7 formed swarm rings on both types of plates. Thus, the inability of *S. typhimurium* to respond to maltose is a property of the first 256 amino acids of Tar, which encompass the periplasmic domain.

Transducer genes from *K. pneumoniae*. The differences in transducer identity and function between *E. coli* and *S. typhimurium* raise a question about the transducer complement of other enteric bacteria. *K. pneumoniae* presents an especially intriguing case, since cells of this species are nonmotile. Twenty phage isolated from a *K. pneumoniae* genomic library restored the ability of *E. coli* strain VB12 to form swarm rings in tryptone soft agar. Lysogens of strain VB12 containing these phage were then screened on serine, aspartate, and maltose swarm plates (data not shown). Five

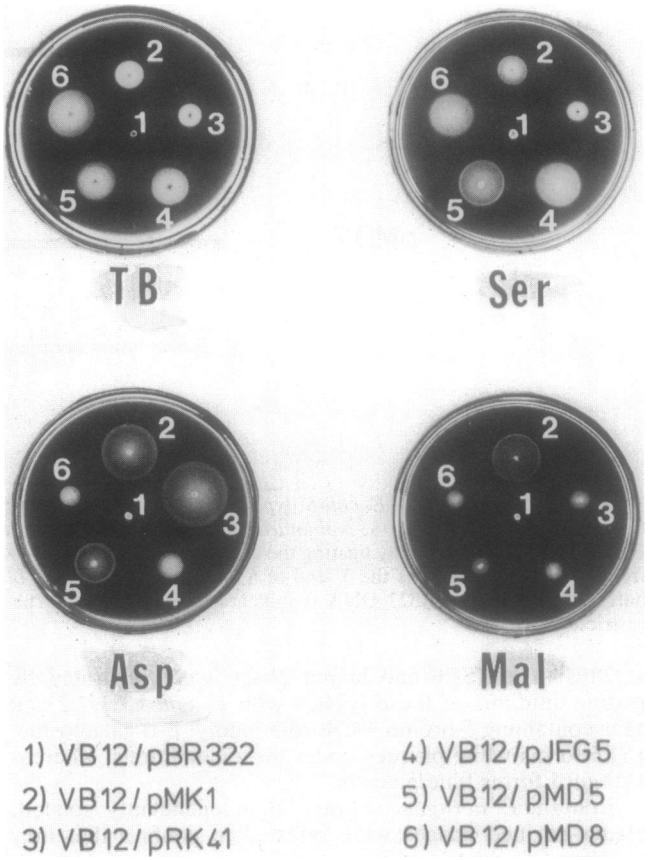

FIG. 3. Chemotactic swarms formed by strain VB12 containing different plasmids. The plasmid in each strain is indicated by the number next to the swarm as follows: 1, pBR322; 2, pMK1; 3, pRK41; 4, pJFG5; 5, pMD5; 6, pMD8. The swarm plates used were tryptone (TB), minimal-serine (Ser), minimal-aspartate (Asp), and minimal-maltose (Mal). All plates contained 50 μg of ampicillin per ml.

phage presumably carry a serine transducer gene, since they confer the ability to form swarm rings in serine soft agar but not in aspartate or maltose soft agar. Fifteen phage did not restore swarm rings with these three attractants. These phage must carry genes for transducers mediating responses to other compounds present in the tryptone extract.

Transducer genes from *E. aerogenes*. Unlike *K. pneumoniae*, *E. aerogenes* is motile. It also responds to many of the same attractants as *E. coli*, suggesting that these two species should have at least some similar transducers. To compare the structure and function of a related transducer from the two bacteria, we set out to clone the *tar* gene equivalent from *E. aerogenes*.

Phage λSE6-M1, isolated from the *E. aerogenes* genomic library, is able to complement the defect in aspartate taxis of strain VB12. DNA prepared from this phage was digested with *Nru*I, and a 5-kb fragment was ligated into the *Nru*I site of plasmid pBR322 to yield pMD5 (Fig. 2). This plasmid and the reference plasmids pBR322, pJFG5 (*E. coli tsr*$^+$), pMK1 (*E. coli tar*$^+$), and pRK41 (*S. typhimurium tar*$^+$) were transformed into *E. coli* VB12, and the chemotactic phenotypes of the transformants were tested on swarm plates (Fig. 3). Plasmid pMD5 restored the ability to form swarm rings with both aspartate and serine, but not with maltose. None of the plasmids restored the ability to form swarm rings with L-prolyl-L-leucine, a good attractant for dipeptide chemotaxis (data not shown).

DNA sequence analysis (described below) revealed that the 5-kb *Nru*I fragment in pMD5 contained two genes that encode transducers. One of these genes, including its promoter, was located on a 2.3-kb *Eco*RI-*Stu*I restriction fragment that was inserted into the *Eco*RI and *Nru*I sites of pBR322 to generate pMD8 (Fig. 2). This plasmid was transformed into strain VB12, where it restored the ability to form swarm rings with serine but not with aspartate (Fig. 3). The transducer gene on plasmid pMD8 was named *tse* (taxis to serine), because it complemented the defect in serine taxis caused by a *tsr* mutation. The transducer gene present on pMD5 but not on pMD8 was named *tas* (taxis to aspartate) because it was required to complement the defect in aspartate taxis caused by a *tar* mutation.

Nucleotide sequence of chemotaxis-related genes from *E. aerogenes*. The nucleotide sequence of the 5-kb *Nru*I restriction fragment from plasmid pMD5 was determined from both strands between two and five times, and the amino acid sequences of the five longest open reading frames were predicted (Fig. 4). Based on the similarity of these sequences to their homologs in *E. coli* (33), three of the open reading frames correspond to *cheW* and fragments of the *'cheA* and *cheR'* genes. The open reading frames corresponding to *tse* and *tas* were identified by their resemblance to transducer genes from *E. coli* and *S. typhimurium*.

Comparison of Che proteins from *E. aerogenes* and *E. coli*. The open reading frame for the 3'-terminal portion of *cheA* from *E. aerogenes* (Fig. 4) codes for 226 amino acids, of which 176 (78%) are identical to residues within the last 226 amino acids of CheA from *E. coli* (33). One stretch of 19 amino acids has only one match, but we do not believe our sequence is in error because neither alternate reading frame in this region gives a better match. Without these 19 residues the percent identity rises to 85% (175 of 207 amino acids).

The 5' region of *cheR* in our sequence contains an open reading frame coding for 100 amino acids starting with the ATG codon at nucleotides 4715 through 4717 (Fig. 4). This codon is preceded by the sequence GAGCT at nucleotides 4707 through 4711, which may serve as a ribosome-binding site. (The C does not match the Shine-Dalgarno consensus sequence.) Alternatively, translation could start at the ATG codon at nucleotides 4769 through 4771. Significant homology between the predicted CheR amino acid sequences of *E. aerogenes* and *E. coli* begins at the glutamine preceding the second methionine residue. Of the next 82 residues, 68 (83%) are identical in the two species. The sequences preceding this glutamine bear little similarity in the two species.

The predicted amino acid sequence of the CheW protein of *E. aerogenes* is 165 residues long and begins with the methionine coded by the ATG triplet at nucleotides 715 through 717 (Fig. 4). This protein is 77% identical to CheW from *E. coli* and 79% identical to CheW from *S. typhimurium* (Fig. 5). The CheW proteins of the latter two species are 92% identical.

Comparison of Tse and Tas with signal transducers from *E. coli* and *S. typhimurium*. The predicted amino acid sequences of Tse and Tas can be aligned with the sequences of four other signal transducers (Fig. 6). The amino acid sequences of the periplasmic domains of the six transducers are compared in Table 2. Tse and Tsr are more like each other than any of the other transducers, being almost as similar as the *E. coli* and *S. typhimurium* Tar proteins. Surprisingly, the periplasmic portion of Tas has a low percent identity with all of the other transducers, exhibiting similarities only in a few, limited regions. The periplasmic portion of Tap diverges even more widely.

DISCUSSION

The properties of *E. coli-S. typhimurium* Tar hybrids suggest that differences in the N-terminal halves of the two proteins (presumably in their periplasmic receptor domains) determine whether they function in maltose taxis. Since *E. aerogenes* carries out both aspartate and maltose taxis (19), the periplasmic domain of its Tar transducer might share structural features with *E. coli* Tar that contribute to its interaction with maltose-binding protein. These features could be recognizable as amino acid sequences present in *E. coli* and *E. aerogenes* Tar but not in *S. typhimurium* Tar. Our interest in making this comparison led us to isolate transducer genes from *E. aerogenes*. As the work progressed, the broader goal emerged of using a comparative approach to study the evolution of the signal transducers.

The nonmotile bacterium *K. pneumoniae* contains at least two transducer genes that can function in *E. coli*. One of them codes for a serine transducer. No defined substrates have been identified for the other *K. pneumoniae* transducers, which overcome the general chemotactic defect of strain VB12.

Why have transducer genes been retained in a nonmotile species? In *E. coli*, the transducer genes are expressed late in the regulatory cascade of the flagellar regulon (2, 20), and their expression is blocked by *fla* mutations that inhibit flagellar synthesis. If comparable mutations have occurred in *K. pneumoniae*, then the transducer genes would not be expressed in that species, and the selective pressure to delete them would be relatively low.

Two transducer genes were found on a 5-kb DNA fragment from *E. aerogenes*. The *tse* gene encodes a serine transducer, since it complements an *E. coli tsr* mutation. The *tas* gene is required to restore aspartate taxis in an *E. coli tar* mutant. Since we do not have a construct in which *tas* is expressed in the absence of *tse*, we have not demonstrated that Tas alone functions as an aspartate transducer, although that is the simplest assumption.

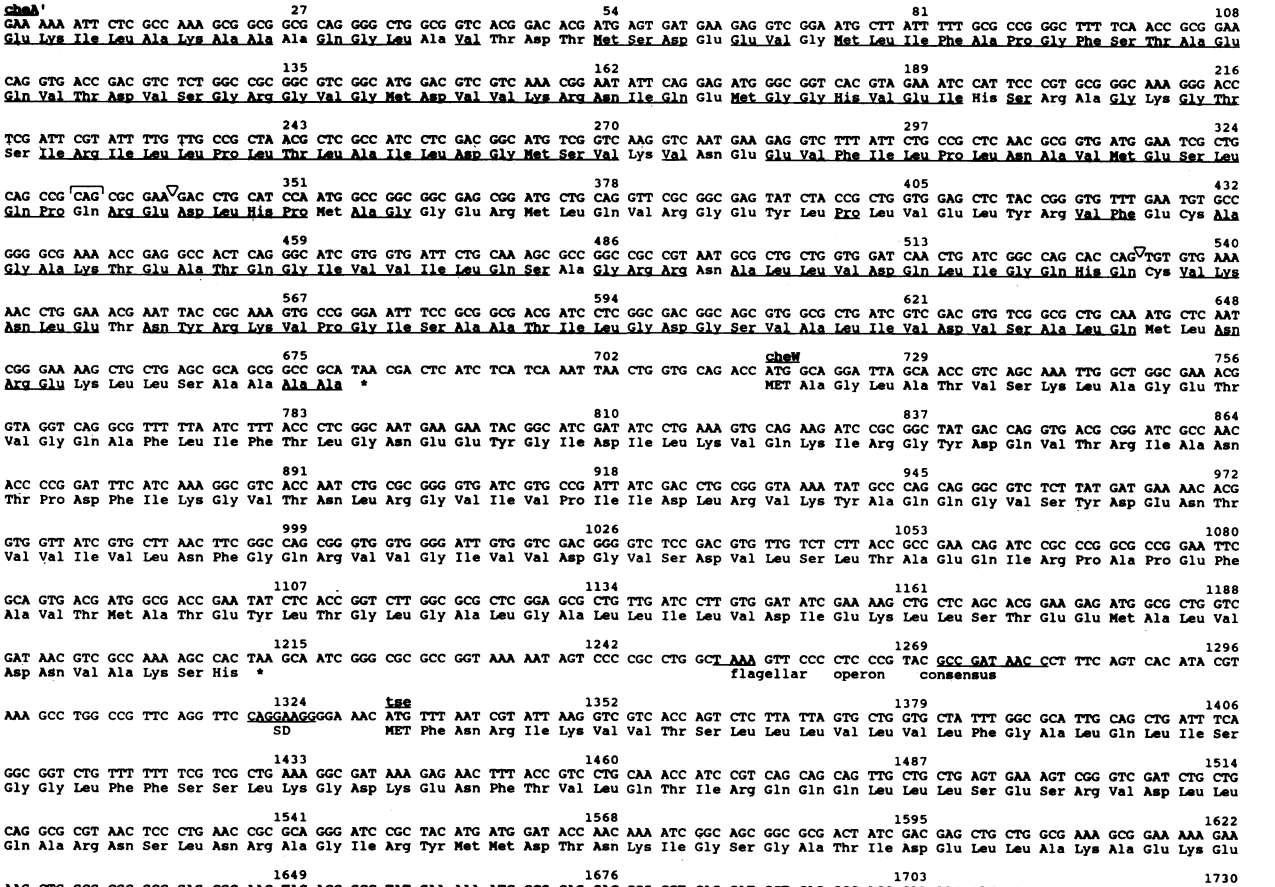

FIG. 4. Nucleotide sequence of the *'cheA-cheW-tse-tas-cheR'* region from *E. aerogenes*. The predicted amino-acid sequences for the longest open reading frames are given below. The amino acids underlined in the *'cheA* and *cheR'* gene fragments are identical with residues in the corresponding genes of *E. coli*. The inverted triangles in *'cheA* indicate single amino acid deletions relative to *E. coli*, and the bracket denotes an inserted amino acid. The underlined sequence designated "flagellar operon consensus" corresponds to the consensus sequence found in the promoters of flagellar operons in *E. coli* (2, 16). The underlined sequences designated SD refer to regions surrounding the predicted Shine-Dalgarno sequences for the *tse* and *tas* genes.

Since *E. aerogenes* responds chemotactically to maltose and synthesizes maltose-binding protein (9), it should have a maltose transducer. Our attempt to isolate this transducer was based on the assumption that it should also be an aspartate receptor. In *E. aerogenes*, however, maltose taxis could be mediated by a minor transducer (like Trg or Tap in *E. coli*) that is unable to restore normal chemotactic behavior to the smooth-swimming VB12 strain. Thus, a search for the gene encoding the maltose transducer of *E. aerogenes* should be repeated with a tsr^+ *tar E. coli* strain, which shows normal swimming behavior but no aspartate or maltose taxis.

The genes on the 5-kb DNA fragment from *E. aerogenes* appear in the order 5′ *cheA-cheW*-promoter-*tse-tas-cheR* 3′ (Fig. 4). This segment spans the 3′ and 5′ ends of the *E. aerogenes* equivalents of the *E. coli mocha* and *meche* operons (33). In *E. coli* the first two genes in the *meche* operon are *tar* and *tap*, whereas in *S. typhimurium tar* is the sole transducer gene in the *meche* operon. The *che* genes are in the same relative location in all three species.

The 36 nucleotides between *cheA* and *cheW* in *E. aerogenes* show no obvious similarity to the corresponding 23-nucleotide regions in *E. coli* or *S. typhimurium*. The untranslated region between *cheW* and the start codon of the first gene in the *meche* operon also varies considerably among the three species. This region contains 122 nucleotides in *E. aerogenes*, 147 nucleotides in *E. coli*, and 243 nucleotides in *S. typhimurium*. The proposed rho-independent site for transcription termination distal to *cheW* in *E. coli* is missing in *E. aerogenes*, as is the perfect 13-base inverted repeat found 3′ to the tandem TAA stop codons of *cheW* in *S. typhimurium*.

The region (underlined in Fig. 4) around the Shine-Dalgarno sequence of the first gene in the *meche* operon is conserved in all three species. So is the consensus sequence (also underlined in Fig. 4) identified in promoters of flagellar operons of *E. coli* (2, 16). Conservation of these regulatory sequences is consistent with the observation that the *tse* and *tas* genes are expressed in *E. coli*. No significant homology was detected between the *tse-tas* (85 base pairs) and *tar-tap* (48 base pairs) or the *tas-cheR* (91 base pairs) and *tap-cheR* (16 base pairs) intergenic regions.

Some regions of the predicted amino acid sequences of the 'CheA and CheR' polypeptide fragments of *E. aerogenes* are more similar to their counterparts in *E. coli* than others (Fig. 4). The highly conserved sequences presumably represent regions of special structural and functional importance. The CheW proteins from *E. aerogenes*, *E. coli*, and *S. typhimu-*

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                                1757                                1784                                1811                                1838
TAC GGC GCG CTG TCG GAG CTG ATC CAG CTG CTG GGC GGA GGG TGT AAA ATC AAC GCC TTC TTC GAC CAG CCG ACG CAA AAA TAC CAG GAC GAT TTC GAG CAG ACC TAT
Tyr Gly Ala Leu Ser Glu Leu Ile Gln Leu Leu Gly Gly Gly Cys Lys Ile Asn Ala Phe Phe Asp Gln Pro Thr Gln Lys Tyr Gln Asp Asp Phe Glu Gln Thr Tyr

                                1865                                1892                                1919                                1946
AAC GCC TAT CTG CAG CAA AAC GGC AAG CTG TAT CAA ATC GCC GTT GAC GCT AGC AAC AGC TCG TAT AGC TCG GCG ATC TGG ACC TTA ATT GTG GTC ATC ATC GTG GTG
Asn Ala Tyr Leu Gln Gln Asn Gly Lys Leu Tyr Gln Ile Ala Val Asp Ala Ser Asn Ser Ser Tyr Ser Ser Ala Ile Trp Thr Leu Ile Val Val Ile Ile Val Val

                                1973                                2000                                2027                                2054
CTG GCG GCG ATC GTC GGC GTG TGG ATG GGT ATC CAC CAT ATC CTG GTG CGC CCG CTG AAC CGC ATG ATA GAA CAC ATC AAA CGG ATC GCG TCC GGC GAT CTG ACC CAG
Leu Ala Ala Ile Val Gly Val Trp Met Gly Ile His His Ile Leu Val Arg Pro Leu Asn Arg Met Ile Glu His Ile Lys Arg Ile Ala Ser Gly Asp Leu Thr Gln

                                2081                                2108                                2135                                2162
CCT ATT CCG GTG ACC AGC CGT AAT GAA ATC GGC GTG CTG GCC GCC AGC CTC AAG CAC ATG CAG AAC GAG CTT ATC GAA ACG GTA AGC GGC GTG CGT CAG GGG GCG GAT
Pro Ile Pro Val Thr Ser Arg Asn Glu Ile Gly Val Leu Ala Ala Ser Leu Lys His Met Gln Asn Glu Leu Ile Glu Thr Val Ser Gly Val Arg Gln Gly Ala Asp

                                2189                                2216                                2243                                2270
CGA TCT ATT GAC GGC GCA TCG GAA AGC GCC GCC GGG AAT AAC GAT CTC TCT TCG CGC ACC GAA CAG CAG GCG GCA GCG CTG GAA GAG ACC GCC GCC AGT ATG GAA CAG
Arg Ser Ile Asp Gly Ala Ser Glu Ser Ala Ala Gly Asn Asn Asp Leu Ser Ser Arg Thr Glu Gln Gln Ala Ala Ala Leu Glu Glu Thr Ala Ala Ser Met Glu Gln

                                2297                                2324                                2351                                2378
CTG ACA GCG ACG GTG AAA CAG AAC GCC GAA AAC GCG CGT CAG GCC AGC CAG CTG GCG CTG AGC GCC TCC GAA ACT GCG CAG AAG GGC GGC AAA GTG GTG GCT AAC GTG
Leu Thr Ala Thr Val Lys Gln Asn Ala Glu Asn Ala Arg Gln Ala Ser Gln Leu Ala Leu Ser Ala Ser Glu Thr Ala Gln Lys Gly Gly Lys Val Val Ala Asn Val

                                2405                                2432                                2459                                2486
GTC GAA ACC ATG CAC GAC ATC GCC AGC AGC TCG CAG AAA ATT GCC GAT ATC ACC GGC GTT ATC GAC GTA ATC GCC TTC CAG ACC AAC ATC CTG GCG CTT AAC GCC GCG
Val Glu Thr Met His Asp Ile Ala Ser Ser Ser Gln Lys Ile Ala Asp Ile Thr Gly Val Ile Asp Val Ile Ala Phe Gln Thr Asn Ile Leu Ala Leu Asn Ala Ala

                                2513                                2540                                2567                                2594
GTA GAA GCG GCG CGT GCG GGT GAA CAA GGT CGC GCG TTC GCG GTC GTG GCG GGC GAA GTC CGC AAC CTG GCG CAG CGC AGC GCC CAG GCG GCC AAA GAG ATC AAA GCG
Val Glu Ala Ala Arg Ala Gly Glu Gln Gly Arg Ala Phe Ala Val Val Ala Gly Glu Val Arg Asn Leu Ala Gln Arg Ser Ala Gln Ala Ala Lys Glu Ile Lys Ala

                                2621                                2648                                2675                                2702
CTG ATC GAA GAC TCG GTC AAC CGT GTG GAT ATG GGC TCG GTA CTG GTG GAA AGC GCC GGC GAC ACC ATG GGC GAT ATC GTC AAT GCG GTC ACC CGC GTG ACC GAC ATC
Leu Ile Glu Asp Ser Val Asn Arg Val Asp Met Gly Ser Val Leu Val Glu Ser Ala Gly Asp Thr Met Gly Asp Ile Val Asn Ala Val Thr Arg Val Thr Asp Ile

                                2729                                2756                                2783                                2810
ATG GGT GAA ATC GCC TCT GCT TCC GAT GAA CAG AGC CGC GGT ATC GAC CAG GTC GGC CAG GCG GTA CGG GAA ATG GAT CGC GTC ACC CAG CAG AAC GCC TCG CTG GTG
Met Gly Glu Ile Ala Ser Ala Ser Asp Glu Gln Ser Arg Gly Ile Asp Gln Val Gly Gln Ala Val Arg Glu Met Asp Arg Val Thr Gln Gln Asn Ala Ser Leu Val

                                2837                                2864                                2891                                2918
GAG GAG TCG GCC TCG GCC GCC GCG CTG GAA GAG CAG GCC AGC CTG CTG ACG CAG TCG GTT GCC GTC TTC CGC CTG AAG TCG GAA GGG CAG GAA GAG TAT AAA GCG CCG
Glu Glu Ser Ala Ser Ala Ala Ala Leu Glu Glu Gln Ala Ser Leu Leu Thr Gln Ser Val Ala Val Phe Arg Leu Lys Ser Glu Gly Gln Glu Glu Tyr Lys Ala Pro

                                2945                                2972                                2999                                3026
GTC AGC AAT AAA ACC GCG CCT GCC GCC ATC GCT ACG CAT AAA AAA ACC AGC GCC AGC GAC TAC CAG GAT AAC TGG GAG ACG TTC TAA CGG GTA CCG GCC GCG ATA CCC
Val Ser Asn Lys Thr Ala Pro Ala Ala Ile Ala Thr His Lys Lys Thr Ser Ala Ser Asp Tyr Gln Asp Asn Trp Glu Thr Phe  *

                                3053                                3080          tas   3105                                3132
TAC ACG CGG CGG CCT CTC TTG CGT TGT AAG CCG GCC GAG ACC GGC TAC CAA GGA GAT TGC T   ATG TTT AAC CGA ATC CGT ATC TCT ACC AGT CTT TTC CTG CTG CTG
                                                              SD                    MET Phe Asn Arg Ile Arg Ile Ser Thr Ser Leu Phe Leu Leu Leu

                                3159                                3186                                3213                                3240
ATC TCC TTT TGC ATC ATG CAA CTG ATC AGC ACC GGG CTC TCT TAC GTC CGG TAC GCG CCG ACA ACC ACA ATC TTG AAC GTA TTA CCG TCA GTA GCC AGC ACG GAT GCG
Ile Ser Phe Cys Ile Met Gln Leu Ile Ser Thr Gly Leu Ser Tyr Val Arg Tyr Ala Pro Thr Thr Thr Ile Leu Asn Val Leu Pro Ser Val Ala Ser Thr Asp Ala

                                3267                                3294                                3321                                3348
CTT AGC CTG AGC TGG GTA TCG CTG TTA CAG GCG AGA AAT ACC CTT AAC CGC GCC GGT ACC CGC GGC GCT GAA GGT GCC GCA GGA GCA GGT GGA AGT GCG CTG ATG GCG
Leu Ser Leu Ser Trp Val Ser Leu Leu Gln Ala Arg Asn Thr Leu Asn Arg Ala Gly Thr Arg Gly Ala Glu Gly Ala Ala Gly Ala Gly Gly Ser Ala Leu Met Ala

                                3375                                3402                                3429                                3456
GCG CCC GCA GCT CGC TGC AAA AAG CCG ATC TCT ATT TTA ACC AGT TCC TCG ACA CGC CTC GCG CGG ATG AGC AGG AAC AGC AGT TGG CCG ATG CCA CGC GTG ATA GCT
Ala Pro Ala Ala Arg Cys Lys Lys Pro Ile Ser Ile Leu Thr Ser Ser Ser Thr Arg Leu Ala Arg Met Ser Arg Asn Ser Ser Trp Pro Met Pro Arg Val Ile Ala

                                3483                                3510                                3537                                3564
ACG AGA ATC TAC GCG CTG CGC GAA CTG ATC GTG TTC CTC GAA AAT CGC AAC CTG CAG GCC TTT ATG GAC CAG CCG ACG CAG AAA ATA CAG GAC CGC TTT GAG GCC GAC
Thr Arg Ile Tyr Ala Leu Arg Glu Leu Ile Val Phe Leu Glu Asn Arg Asn Leu Gln Ala Phe Met Asp Gln Pro Thr Gln Lys Ile Gln Asp Arg Phe Glu Ala Asp

                                3591                                3618                                3645                                3672
TTT GTG CAA TAT CTG CAG CTG GCG AAG GCG ACG ACC GAT GAA GCC AGC GCC TCC AGC CAG CAG GCC TAC GCG TTG GTT CCA ATC TGG TTG GTT GCC GGG GCG GTG CTG
Phe Val Gln Tyr Leu Gln Leu Ala Lys Ala Thr Thr Asp Glu Ala Ser Ala Ser Ser Gln Gln Ala Tyr Ala Leu Val Pro Ile Trp Leu Val Ala Gly Ala Val Leu

                                3699                                3726                                3753                                3780
ATG CTC TTG GTA GTC ACC CTC AGC GCG ATG TGG TGG CTG CGA ACC ATG CTG GTG CAG CCG CTG AAT ATT ATT CGC GGC CAT TTC GAA CGT ATC GCC AGC GGC GAT CTT
Met Leu Leu Val Val Thr Leu Ser Ala Met Trp Trp Leu Arg Thr Met Leu Val Gln Pro Leu Asn Ile Ile Arg Gly His Phe Glu Arg Ile Ala Ser Gly Asp Leu

                                3807                                3834                                3861                                3888
TCC GCA CCG ATT GAG GTC TAT GGG CGT AAC GAA ATC AGC CAG CTA TTT GCC AGC CTG CAG CGC ATG CAA CAG TCG CTG ATT GGT ACC GTC GGC GCG GTG CGC GAC GGC
Ser Ala Pro Ile Glu Val Tyr Gly Arg Asn Glu Ile Ser Gln Leu Phe Ala Ser Leu Gln Arg Met Gln Gln Ser Leu Ile Gly Thr Val Gly Ala Val Arg Asp Gly

                                3915                                3942                                3969                                3996
GCG GAG TCG ATT CTT ATC GGC CTG CAG GAG ATT GCC GAA GGC AAC AAC GAT CTC TCC TCG CGC ACC GAA CAA CAG GAC CGT TCG CTG GAA GAG ACC GCC GCC AGT ATG
Ala Glu Ser Ile Leu Ile Gly Leu Gln Glu Ile Ala Glu Gly Asn Asn Asp Leu Ser Ser Arg Thr Glu Gln Gln Asp Arg Ser Leu Glu Glu Thr Ala Ala Ser Met

                                4023                                4050                                4077                                4104
GAG CAG CTA ACG GCG ACG GTA AAA CAG AAT GCC GAT AAC GCC CGC CAG GCA TCG CAG CTG GCG CGT GAT GCC TCC TCC ACC GCC GCC AAA CGG ACG CTG GCG GAT GAC
Glu Gln Leu Thr Ala Thr Val Lys Gln Asn Ala Asp Asn Ala Arg Gln Ala Ser Gln Leu Ala Arg Asp Ala Ser Ser Thr Ala Ala Lys Arg Thr Leu Ala Asp Asp

                                4131                                4158                                4185                                4212
GTG GTC ACC ACG ATG CAC GAT ATC GCC AAC AGT TCG CAA AAA ATC GGC GCG ATT ACC AGC GTC ATC GAC GGC ATC GCT TTC CAG ACC AAT ATT CTG GCG CTT AAC GCG
Val Val Thr Thr Met His Asp Ile Ala Asn Ser Ser Gln Lys Ile Gly Ala Ile Thr Ser Val Ile Asp Gly Ile Ala Phe Gln Thr Asn Ile Leu Ala Leu Asn Ala

                                4239                                4266                                4293                                4320
GCG GTG GAG GCG GCG CGC GCC GGT GAA CAG GGG CGC GGC TTT GCG GTG GTG GCC GGT GAA GTG CGC AAC CTG GCC AGC CGC AGC GCC CAG GCG GCA AAA GAA ATC AAG
Ala Val Glu Ala Ala Arg Ala Gly Glu Gln Gly Arg Gly Phe Ala Val Val Ala Gly Glu Val Arg Asn Leu Ala Ser Arg Ser Ala Gln Ala Ala Lys Glu Ile Lys

                                4347                                4374                                4401                                4428
CTG CTG ATC GAT GAA TCC GTC AGC CGC GTC AAA CAC GGC TCG GTG CTG GTG GAA AAT TCC GGC GCC ACC ATG CAG GAC ATC GTG CGC TCG GTC ACC CGG GTC ACC GAC
Leu Leu Ile Asp Glu Ser Val Ser Arg Val Lys His Gly Ser Val Leu Val Glu Asn Ser Gly Ala Thr Met Gln Asp Ile Val Arg Ser Val Thr Arg Val Thr Asp

                                4455                                4482                                4509                                4536
ATC ATG GGC GAA ATC GCC TCC GCC TCC GAT GAG CAG AGC CGC GGC ATC GAG CAG GTG ACG CAG GCG TTA CCC AGA TAT CAG GTC ACG CAA CAG AAC GCC GCC CTG GTG
Ile Met Gly Glu Ile Ala Ser Ala Ser Asp Glu Gln Ser Arg Gly Ile Glu Gln Val Thr Gln Ala Leu Pro Arg Tyr Gln Val Thr Gln Gln Asn Ala Ala Leu Val

                                4563                                4590                                4617                                4644
GTG GAA TCG GCT TCC GCC GCC GCT GCG CTG GAA GAG CAG GCG ATT ACC CTC GCC GAT GCA GTG GCG GTA TTC CGT CTG GCG GAC GAC TAA TTG TCG CGC CGG ATA TAC
Val Glu Ser Ala Ser Ala Ala Ala Ala Leu Glu Glu Gln Ala Ile Thr Leu Ala Asp Ala Val Ala Val Phe Arg Leu Ala Asp Asp  *

                                4671                                4698                          cheR'  4723                                4750
CTA TCC AGG TAA AGG AGA CCC GGT GGA TTG TCA AAC CGC ATT AAA CGC TCG TAT TTG CCG GTG AGC TGG C   ATG AAG CAA GAC GAC ATC AAC GCG GCG CGT GAA AGC
                                                                                               MET Lys Gln Asp Asp Ile Asn Ala Ala Arg Glu Ser

                                4777                                4804                                4831                                4858
GGA TCG GCC GTG GCG CAG ATG GCC CAG CGT CTG CCG CTC TCC GAC GCG CAT TTT CGC CGC ATC AGC CAG CTT ATC TAT CAG CGC GCC GGG ATC GTG CTG GCG CCG CAT
Gly Ser Ala Val Ala Gln Met Ala Gln Arg Leu Pro Leu Ser Asp Ala His Phe Arg Arg Ile Ser Gln Leu Ile Tyr Gln Arg Ala Gly Ile Val Leu Ala Pro His

                                4885                                4912                                4939                                4966
AAG CGC GAG ATG GTG TAC AAC CGG CTG GTG CGC CGT TTA CGT CTG CTG GGC ATT CAT GAT TTC GGC GAC TAC CTG GCG CTG CTG GAA AGC GAC CCG CAC AGC GCC GAG
Lys Arg Glu Met Val Tyr Asn Arg Leu Val Arg Arg Leu Arg Leu Leu Gly Ile His Asp Phe Gly Asp Tyr Leu Ala Leu Leu Glu Ser Asp Pro His Ser Ala Glu

                                4993                                5015
TGG CAG GCG TTT ATC AAT GCG CTG ACC ACC AAC CTG ACC GCC TTT TTT C
Trp Gln Ala Phe Ile Asn Ala Leu Thr Thr Asn Leu Thr Ala Phe Phe
```

FIG. 4—*Continued*

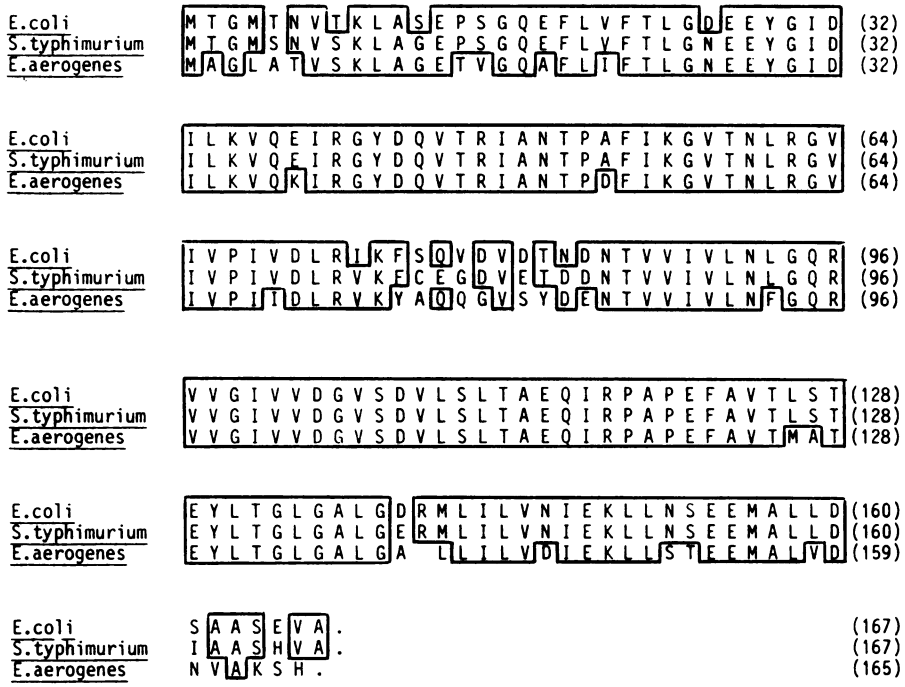

FIG. 5. Comparison of the predicted amino acid sequences of CheW from *E. coli* (33), *S. typhimurium* (43), and *E. aerogenes*. Identical residues are boxed. The β-α-β structural motif (45) extends over residues 128 through 148 and 155 through 160.

rium also have a pattern of conserved and variable regions (Fig. 5). CheW has been proposed to contain a nucleotide-binding site (43). An 11-residue consensus sequence that determines the β-α-β motif of the binding site has been described (45). All three species have the identical amino acid at each of the 11 positions, and 10 of the 11 residues conform to the consensus. Other highly conserved segments of CheW may correspond to sites of interaction between CheW and the signaling domains of the transducers (P. Ames, J. Chen, C. Wolff, and J. S. Parkinson, Cold Spring Harbor Symp. Quant. Biol, in press).

Tse and Tas have the typical structural features of signal transducers (Fig. 6): a positively charged N terminus (residues 1 through 6) followed by a hydrophobic membrane-spanning region (residues 7 through 30), a hydrophilic periplasmic domain (residues 31 through 190), a second hydrophobic membrane-spanning region (residues 191 through 210), and a hydrophilic cytoplasmic domain (residue 210 through the C terminus). Of 187 residues that are identical in the six transducers in Fig. 6, 168 are within the cytoplasmic domain and 145 are within a stretch of 236 amino acids (residues 280 through 516 in Tsr).

The K1 and R1 tryptic peptides of Tsr (residues 295 through 317 and 483 through 507) contain the sites of covalent methylation (19). Within the region corresponding to K1, Tse and Tsr are identical at 22 of 23 residues and Tas and Tsr are identical at 21 of 23 residues. The same three methylation sites, which are Glu-Glx sequences, are present in Tse and Tas as well as in Tsr and Tar. Within R1, Tse and Tsr are identical at 21 of 25 residues and the two methylation sites are present. Tas and Tsr are identical at 20 of 25 residues, but one of the methylation sites may be absent in Tas due to the replacement of the Glu-Glu sequence at residues 492 and 493 of Tsr with Val-Glu in Tas.

Another highly conserved stretch runs from residues 360 through 407 of Tsr. The same 48 residues are present in Tar, and there is only one difference in Tap. Tse and Tas share 44 of the 48 residues with Tsr. In Trg, only 37 of these 48 residues are identical to Tsr (5), underscoring the proposed early divergence of Trg from the other transducers. The strong conservation of this region and of the K1 and R1 peptides emphasizes their importance in signaling and adaptation, respectively (Ames et al., in press).

The Tse and Tsr proteins are 60% identical in their periplasmic domains (Fig. 6 and Table 2). Even their cytoplasmic domains resemble one another more closely than they do those of other transducers. The periplasmic domain of Tas bears little resemblance to that of any other known transducer, including Tap, which like Tas is the product of the second gene in a *meche* operon, or Tar, which is a functional analog of Tas. Given the dissimilarity of Tar and Tas, it is not surprising that Tas does not participate in maltose taxis.

The amino acid transducers Tsr, Tse, Tas, and Tar share three sites that are partially or wholly absent in Tap and Trg: (i) Arg at residues 64, 69, and 73 (Arg-64 is present in Trg); (ii) Glu-Leu-Ile at residues 138 through 140; (iii) Gln-Pro-Thr-Gln at residues 154 through 157 (Pro-155 and Gln-157 are present in Tap). Genetic studies indicate that some of these residues are important in chemoreception. *E. coli* mutants in which Arg-64, Arg-69, or Arg-73 of Tar is replaced by other residues have a drastically reduced ability to carry out aspartate taxis, and the apparent affinity for aspartate is reduced by a factor of 10^2 or more (46). Furthermore, a mutation affecting the Arg-64 residue of *E. coli* Tsr greatly reduces the ability of cells to sense serine (25). The three Arg residues may form a positively charged pocket that interacts with a common element of amino acids, such as their α-carboxyl group (25, 46; Ames et al., in press). Other data suggest that Thr-156 of Tsr and Tar is important for serine (25) and aspartate (C. Wolff, personal communication) sensing.

Tse (with 557 residues) and Tas (with 510 residues) are the largest and smallest transducers, respectively, known in the

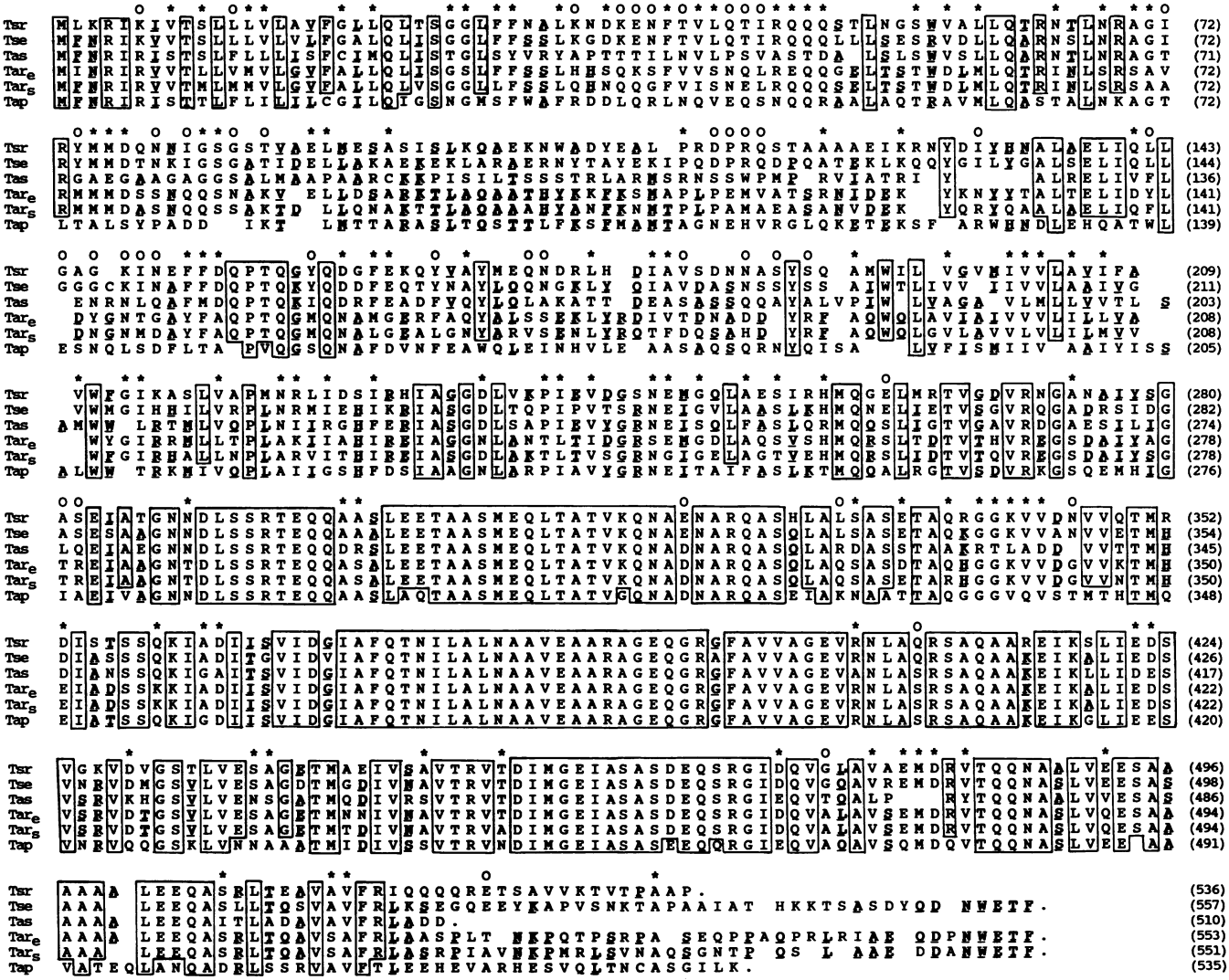

FIG. 6. Comparison of the predicted amino acid sequences of signal transducer proteins Tsr (*E. coli*), Tse (*E. aerogenes*), Tas (*E. aerogenes*); Tar_E (*E. coli*), Tar_S (*S. typhimurium*), and Tap (*E. coli*). The sequences are aligned to give the maximal number of residue identities among the six transducers, using the corrected sequence of Tsr (12, 25, 27). This alignment introduces some gaps, most of which can reasonably be explained by the deletion or insertion of codons in particular lineages. The alignment for a sequence (covering residues 194 through 212 of Tsr) that includes most of the second membrane-spanning region contains the most gaps and has the lowest qualitative confidence level. Residues identical in all six transducers, or in all except Tap, are boxed. Symbols: *, positions at which residues are identical in Tsr and Tse and at least one other transducer; O, positions at which residues are identical in Tse and Tsr but different in the other transducers. For all other positions, residues that are the same in two or more transducers are printed boldface and underlined. The residue count for each transducer is indicated within parentheses at the end of each line.

enteric bacteria. Most of this variation in length occurs at the extreme C termini of the proteins, which are among the most highly variable segments (Fig. 6). It is striking that the C termini of Tse and Tar are of about the same length and that the last five residues are identical. These features may be correlated with the location of *tse* and *tar* at the 5′ end of the *meche* operon. Tsr, encoded by a gene located elsewhere on the chromosome, has a very different C terminus, although the Tsr protein is otherwise homologous with Tse.

Within the enteric bacteria there appear to be at least five evolutionary lines among the transducers. Trg, which looks least like the others, may represent the fusion of the N terminus of a membrane transport protein with a C-terminal domain common to all transducers (5). The remaining four groups (Tsr-Tse, Tar, Tas, and Tap) are similiar enough that they probably arose by mutational divergence of intact genes. Their progenitor may have been an amino acid receptor of rather low specificity. Crucial features of the binding site may be conserved in the three Arg residues and the sequences at positions 138 through 140 and 154 through 157. Subsequent gene duplication would have allowed individual transducers to evolve a higher affinity for certain substrates. The interaction with substrate-binding proteins (maltose-binding protein for Tar, dipeptide-binding protein for Tap) could have elaborated later.

E. coli, *S. typhimurium*, and *E. aerogenes* all have one or two transducer genes in their *meche* operons, but the genes occur in three different combinations. It may be that all transducers other than Trg evolved at this locus and that some of the genes were then deleted or moved to new sites by chromosome rearrangements. Alternatively, duplicated genes may have been redistributed on the chromosome

TABLE 2. Sequence identities in the periplasmic domain of chemotactic signal transducers[a]

Amino acid	No. (%) of identical residues in:				
	Tse	Tas	Tar_E	Tar_S	Tap
Tsr	100 (60)	43 (26)	53 (32)	51 (31)	32 (19)
Tse		50 (30)	52 (31)	50 (30)	27 (16)
Tas			35 (21)	33 (20)	29 (17)
Tar_E				113 (68)	35 (21)
Tar_S					35 (21)

[a] The amino acid sequences for the six transducers, aligned as in Fig. 6, were compared for the region encompassing residues 31 to 193 of Tsr. The region contains 167 residue positions, although no single transducer has an amino acid at each position. The Table presents the number of residue identities over these 167 positions in pairwise combinations of transducers. Since the deletion or insertion of a codon can occur during gene evolution, the absence of an amino acid at the same position in two or more transducers was also scored as an identity. The percent identity is given in parentheses; it would be altered only slightly if shared gaps were not counted as identities.

before their evolutionary divergence. Genes evolved elsewhere could then have reinvaded the *meche* operon by recombining with the resident transducer genes. The study of transducer evolution will be enhanced by information from a wide taxonomic spectrum of bacteria.

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